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Phytochemical and High-Performance Liquid Chromatography Analysis of Extract of *Vernonia cinerea*

Ruchi Acharya[1*], Bhawna Sharma[1], Ruchi Singh[1], Prabhat Jain[2]

[1] Career College, Kalibadi Rd Opp to Dussehra ground BHEL, Sector A, Govindpura, Bhopal, MP 462023

[2] Scan Research Laboratories, Sector A H No. 109, J K Road, Indrapuri, Bhopal , MP 462023

ABSTRACT

The present study aims to screen and quantify hydroalcoholic extract for phytochemical content and HPLC profiles for standardization. HPLC was carried out using a RP-C18 analytical column with a mobile phase composed of acetonitrile: methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL min-1. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm. Phytochemical screening of the extract showed the presence of flavonoids, amino acids, carbohydrates and protiens in hydroalcoholic extract. Quantification of total flavonoids showed that hydroalcoholic extract of *Vernonia cinerea* had flavonoid content 0.547 mg/100mg equivalent to quercetin. The data presented here could be used for the standardization of hydroalcoholic extract of *Vernonia cinerea*, either for future studies or in herbal drug formulations.

Keywords: *Vernonia cinerea*, HPLC profiling, Phytochemical analysis, Hydroalcoholic extract.

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***Address for Correspondence:**

Ruchi Acharya, Career College, Kalibadi Rd, Opp to Dussehra ground BHEL, Sector A, Govindpura, Bhopal, MP 462023

INTRODUCTION

Plant extracts were used in folk medicine in the form of tinctures, infusions and decoctions or essential oils, as a cure and treatment of diseases all over the world. The most common studied plants for their therapeutic potential are considered the spices and medicinal herbs. Although they are extensively used for the design and development of new drugs in human medicine, plant antimicrobial compounds are also a promise for future plant disease controlling agents. The use of plant products as antimicrobial agents is an ancient idea[1], but the researches in the area are gaining attention lately. As a response to the acquired pathogens resistance to antibiotics existing on the market, new alternatives should be designed for the treatment of infectious diseases. It is therefore desirable to explore the potential of plant extracts for the development and design of new antimicrobial agents[2,3], as this could be a solution for both medical and phytopharmaceutical industry. Plants contain natural bioactive compounds such as secondary metabolites and antioxidants. The medicinal plants used as traditional medicine all over the world are rich in secondary metabolites[4]. The traditional medicine all over the world is nowadays revealed by an extensive activity of researches on different plant species and their therapeutic principles. Plants contain phytochemicals with various bioactivities including antioxidant, anti-inflammatory and anticancer activities. The present study aims to screen and quantify hydroalcoholic extract for phytochemical content, and HPLC profiles for standardization. *Vernonia cinerea* (Family: Asteraceae) is a terrestrial annual erect herb. *V. cinerea* is an important medicinal plant having application in abortion, cancer and various gastrointestinal disorders[5]. Toxicity study of the plant on mice was carried out but the results were inadequate for definite conclusion[6]. Chloroform extract of stem-bark and leaves of *Vernonia cinerea* showed diuresis property but methanolic extract exhibited antidiuresis[7]. Both polar and non-polar fraction of the plant extract showed analgesic, antipyretic and anti inflammatory effect[8]. Polar extract of *V. cinerea* is found to have antidiarrhoeal activity[9] but there is no study on non-polar fraction. Antibacterial [10]and anti larval activity against filarial vector[11]. Carbon tetrachloride fraction of methanolic extract possesses significant antioxidant properties[12].

MATERIALS AND METHODS

Plant material collection

The plant has been selected on the basis of its availability and folk use of the plant. The plant *Vernonia cinerea* (Root)

was collected from local area of Bhopal (M.P.) in the month of Jan, 2018.

Processing of the plant material

Drying of fresh plant parts was carried out in sun but under the shade. Dried *Vernonia cinerea* (Root) was preserved in plastic bags and closed tightly and powdered as per the requirements.

Extraction procedure

Dried powdered of *Vernonia cinerea* (Root) was subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. 50 gm powdered of Vernonia *cinerea* (Root) was extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40˚C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of powder drug taken}} \text{x } 100$$

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [13,14].

Total flavonoid content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of extract was dissolved in 10 ml methanol and filter. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm[15].

Identification of marker compound (Quercetin) by HPLC

Reagents and chemicals

Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India.

Instrumentation

A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λmax. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C_{18} (250 X 4.6 mm, 5µm) column, a Data Ace software.

Chromatographic conditions

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 ml/min. A small sample volume of 20 µl was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm.

Determination of λmax

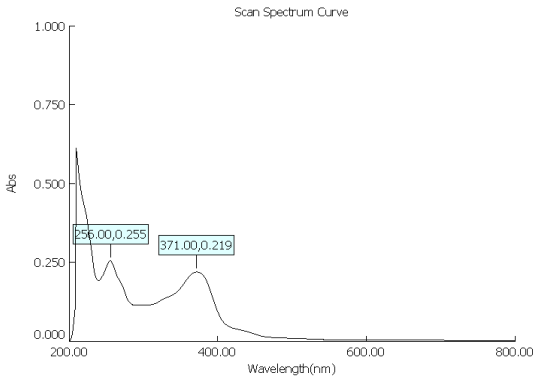

Figure 1: Determination of λmax

Selection of separation variable

Table 1: Selection of separation variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane C_{18})
Mobile Phase	
Acetonitrile	50
Methanol	50
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	256 nm
Retention time	2.60± 0.5 min

Preparation of standard stock solution

10mg of quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of working standard solution

From stock solutions of quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Analysis of extracts

Sample Preparation

10 mg extract was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using Whatmann filter paper no. 41 and then sonicated for 10 min.

RESULTS AND DISCUSSION

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using hydroalcoholic as solvents are depicted in the Table 2.

Table 2: % Yield of hydroalcoholic extract of *Vernonia cinerea*

S. No.	Plant material	% Yield (W/W)
1.	**Root**	3.5

Phytochemical screening of the extract showed the presence of flavonoids, amino acids, carbohydrates and proteins in hydroalcoholic extract table 3.

Table 3: Result of phytochemical screening of hydroalcoholic extract of *Vernonia cinerea*

S. No.	Constituents	Root
1.	Alkaloids	-
2.	Flavonoids	+
3.	Diterpenes	-
4.	Phenolics	-
5.	Amino Acids	+
6.	Carbohydrate	+
7.	Proteins	+
8.	Saponins	-

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.040X + 0.009, R^2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance. The result are shown in table 4,5 and fig 2.

Table 4: Calibration curve of quercetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

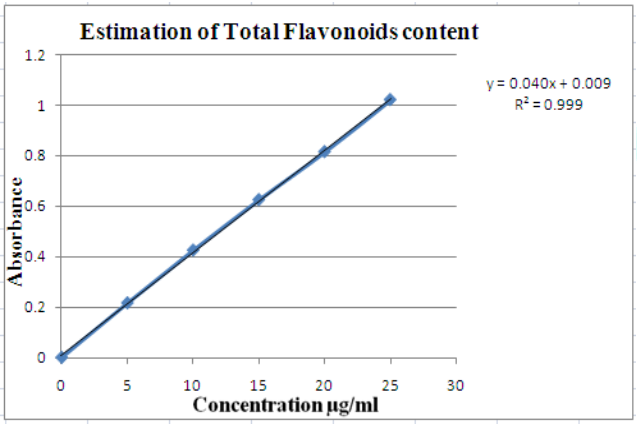

Figure 2: Graph of estimation of total flavonoids content

Table 5: Estimation of total flavonoids content of *Vernonia cinerea*

S. No	Plant material	Total flavonoids Equivalent to Quercetin mg/ 100 mg of extract
1.	**Root**	0.547

A reverse phase C-18 column equilibrated with mobile phase methanol: acetonitrile (50:50, v/v) was used. Mobile phase was filtered through Whatmann filter paper and degassed. Mobile phase flow rate was maintained at 1 ml/min and effluents were monitored at 256 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 10 min. The sample solution was chromatographer and a concentration of quercetin in extract sample was found out using regression equation. Each of the standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Table 6: Preparation of calibration curve

S. No.	Conc.	Mean AUC
1.	0	0
2.	5	110.469
3.	10	222.814
4.	15	323.334
5.	20	440.478
6.	25	562.082

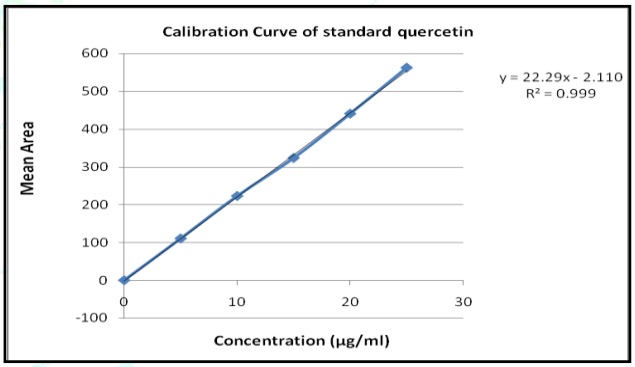

Figure 3: Calibration curve of the quercetin

Table 7: Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range µg/ml	Correlation efficient	Slope	Intercept
Quercetin	5-25	0.999	22.29	2.110

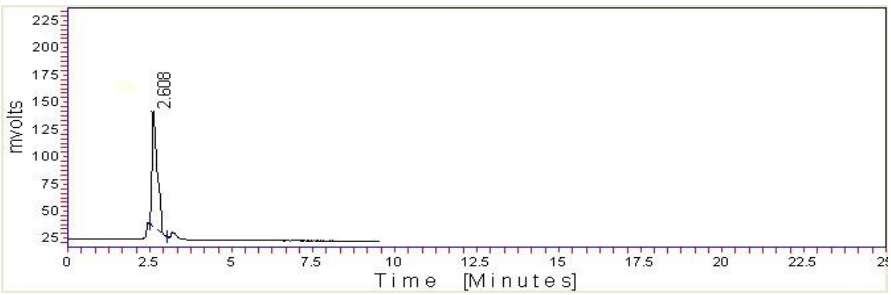

Figure 4: Chromatogram of standard quercetin

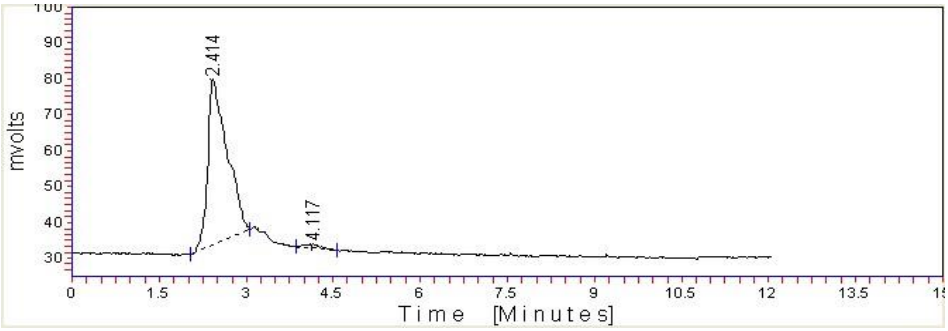

Figure 5: Chromatogram of hydroalcoholic extract

Table 8: Quantitative estimation of quercetin in hydroalcoholic extracts

S. No.	Extract	RT	Area	% Assay
1.	***Vernonia cinerea***	2.414	1018.016	4.576

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities. The results of phytochemical analysis of the extract are summarized in Table 3. The results revealed the presence of medicinally active constituents such as flavonoids, amino acids, carbohydrates and proteins. HPLC fingerprinting analysis summarized in Figure 4 & 5 and showed peaks of the significant area. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the plants. The presence of some of these compounds has also been confirmed to have various pharmacological activities. The study also established quality control parameters of the plant including analytical and phytochemical standardization. In spite of the numerous medicinal of the medicinal plant, the herbal industry suffering many problems regarding quality control standardization of plant material, thus the present investigation of *Vernonia cinerea*. Plant carried out to establish a pharmacognostic and high-performance liquid chromatography (HPLC) fingerprint profile of the plant.

CONCLUSION

Due to the growing interest in herbal drug formulations, standardization of plant extracts using chromatographic techniques and phytochemical quantification have been developed. Current investigation involves quality control characterization of plant *Vernonia cinerea*. The plant extract evaluated for phytochemical and chromatographic analysis. *Vernonia cinerea* have been recently reported to possess various pharmacologic activities, as well as nutritional content. The data presented here could be helpful in standardizing extracts of these plants.

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